Title: The Occurrence and Potential Health Risk of Microcystins in Drinking Water of Rural Areas in China.

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Grant Support: This research was supported by grants from National Key R&D Program of China (No.2017YFC1600200), National Natural Science Fund Committee (No.81273035, 81773379 & 81630088), Shanghai Municipal Health Bureau Three Years Action Plan (No. 08GWD14), and Leadership Project (No. 2017) and Natural High-Technology R&D 863 Program (No.2013AA065204).

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Disclosures: The authors declare they have no actual or potential competing financial interests. The authors have no conflicts to declare.

Abstract:

Large-scale use of nitrogen and phosphorus fertilizers in agricultural production, environmental pollution and climate warming cause frequent algal blooms and the generation of algal toxins in water bodies in China. Algal pollution is increasing and microcystins (MCs) are detectable in both surface and ground water in China at sub-μg/L and μg/L levels. Toxicological studies show that microcystins have hepatic and renal toxicity, genotoxicity, tumor-promoting effects, neurotoxicity, reproductive and developmental toxicity. Epidemiological evidence from China further reveals that chronic exposure to MCs through drinking water and liver cancer are positively correlated and demonstrate that MCs in drinking water are a main risk factor in liver cancer. Effectively controlled water pollution, reduced sewage discharge, and enhanced wastewater treatments are pivotal measures to control algal pollution and toxins in the drinking water of rural China.

Key words: Algae; Carcinogenicity; China; Developmental toxicity; Drinking water; Genotoxicity; Hepatic toxicity; Liver cancer; Microcystins; Neurotoxicity; Renal toxicity; Reproductive toxicity

1. Occurrence of Microcystins in China

Much industrial, agricultural and domestic sewage is discharged into water bodies in China, resulting in a significant deterioration in water quality. After 2000, the water quality in the Seven Key River Systems of China was significantly affected. In 2012, only 31.1% of river sections in the key river systems reached national water quality III. Total phosphorus and total nitrogen are important indicators that have effects on water quality. Rich nitrogen or phosphorus in a water body is an important factor affecting eutrophication and algal blooms. Data from the China State Environmental Protection Agency showed that continuous pollution has made water bodies, especially lakes and reservoirs, subject to moderate or severe eutrophication. 10% of key lakes in China are moderately or heavily eutrophicated. Water blooms occur every summer because of the proliferation of algae, which has become one of the most important factors affecting quality of water bodies and drinking water. The algal outbreak that occurred in Lake Taihu in Jiangsu Province in 2007 caused the closure of water plants, which had a serious impact on the daily life of local residents.

The large-scale use of nitrogen and phosphorus fertilizers in agricultural production, environmental pollution and climate warming caused by wastewater discharge during industrial production are important factors leading to algal blooms and the generation of algal toxins. In the past, algal blooms mainly occurred in South China. However, environmental pollution and climate warming have caused algal pollution to move northward. In recent years, algal blooms in Northern China have also become more frequent. In ditch ponds and irrigation channels in rural areas of China, the problem of algal pollution has become increasingly prominent. The algal toxin pollution level in the ditch pond water in some areas is similar to Qidong, Jiangsu, which is a well-known high incidence area of liver cancer in China.

Algal toxins in water can be divided into soluble toxins and intracellular algal toxins. Soluble toxins are secreted by algal cells or released when a cell dies, and intracellular algal toxins are toxins in the algal cells. Although there are more than 90 species of microcystins (MCs) in water, only microcystin-LR (MC-LR) is included in the National Standards for Water Environment and Standards for Drinking Water Quality. Some studies have also focused on the pollution level of several algal toxins with high concentration and relatively high toxicity in water, such as MC-RR, -YR, -LW, and -AF, but such studies are limited, and therefore, data about those toxins' exposure level is fragmentary. In general, algal pollution caused by water pollution is increasing, and algal toxins can be detected in most water bodies in China. Algal toxins are often detected in drinking water because current conventional water treatments cannot remove algal toxins completely.

Algal toxins in ground water are mainly released by dead algal cells; they infiltrate into the ground water and pollute it. When a water bloom occurs, the dissolved oxygen level in the water bodies drops rapidly, causing algae to die of oxygen deficiency, the algae cells to rupture, and algal toxins to be released into the water body. The released toxins will contaminate ground water during the ground water recharge and migration process. In rural China, ground water is the main source of water. In many areas, ground water is directly used as drinking water, therefore people can be exposed to algal toxins through their drinking water. In general, the level of aquatic algal toxins ranges from a few hundred nanograms to tens of micrograms per liter. Algal toxins in water bodies are higher than well water and much higher than tap water. Algal toxins in shallow ground water are higher than deep ground water.

Lake Taihu is China's third largest freshwater body, and cyanobacterial blooms occur frequently. In 2014, Su et al measured microcystins in the Taihu area of Jiangsu and found that the main types of algal toxins were MC-LR, MC-RR and MC-YR, with average concentrations of 4.69, 4.23 and 2.01 μg/L, respectively. Chen et al. (1996) found that the average levels of MCs in ditch-pond water and river water of Haimen, Jiangsu Province were 0.101 and 0.160 μg/L, and the highest levels were 0.300 and 1.558 μg/L, respectively. And they also found that the average levels of MCs were 0.068 and 0.048 μg/L in shallow and deep well water there, respectively. Yang et al (2016) found that the concentration of MCs in ground water of Lake Chaohu ranged from <0.1 to 1.07 μg/L, and their concentrations correlated with the distance from the lakeshore. In 2008, Dai et al monitored MCs in the Beijing Guanting Reservoir and detected MC-LR in the water. The isolation rate was 100%, and its concentration ranged from 0.21 to 1.15 μg/L. Lin's research group (2005) determined that the MCs in the reservoir waters in Guangdong Province were dominated by MC-RR. In 2004, MCs were found in some key reservoirs and lakes in Guangdong through enzyme-linked immunosorbent assays. The average concentrations of MCs in Dongjiang River Basin, Beijiang River Basin, Pearl River Delta, Yangxi Reservoir in the eastern coast of Guangdong, Hedi Reservoir in the western coast of Guangdong, and Xinghu Lake were 0.01, 0.02, 0.176, 0.295, 0.102, and 0.371 μg/L, respectively. Dong et al (1998) detected source water and factory water in several areas of Jiangsu, and MCs in source water ranged from 0.281 to 35.300 μg/L, which was much higher than in factory water (<0.020-1.4 μg/L). Liu et al (2011) found that the concentration of free algal toxins in the main lakes of Wuhan was 0.0146-0.1212 μg/L. From March 1995 to February 1996, Xu et al (2000) monitored MCs in Donghu Lake and a fish pond in Wuhan, and the concentration of MCs ranged from 0.10-0.30 μg/L.

2. Toxicity of Microcystins

2.1 Hepatic and Renal Toxicity

Liver is the main target organ of microcystins. After entering into human body, MCs are stored in the liver, and cause severe liver cell damage, with the clinical features of liver inflammation, liver cell structure disruption and liver cell apoptosis. In several cases, this can lead to liver failure. Studies have shown that dosing of microcystins into rats can cause acute poisoning; and autopsies of the rats showed liver swelling, hyperemia and necrosis. Histopathological observation showed changes such as hepatocyte rounding, necrosis and hyperemia, breakdown of the sinusoidal endothelium and hepatic architecture, cell junction loosening, cell death, swelling of the intracellular membrane system, loss of desmosome and tonofilament, alteration of the microfilament network and cytomorphosis.

Collapsed capillary tufts of glomeruli could be observed in rats treated with MC-LR (10 μg/kg i.p.). In addition, proximal tubules, distal tubules and glomerular vascular became wider, red blood cells increased in the lumen, tubular epithelial cells were often desquamated or entirely missing, vacuolization of the cytoplasm appeared in tubular epithelial cells, and intercellular space lymphocyte infiltration appeared. Rats given MCs intravascularly exhibited decreased sodium reabsorption in renal tubules, impaired renal function and proteinuria after only 90 min.

2.2 Neurotoxicity

Animal experiments showed that MCs can accumulate in the brain, and cross the blood brain barrier to cause changes on nerves and brain. Using MC − immunodetection methods to measure brain cells of rats which had been exposed to MCs for 48 hours, results showed that brain cell levels of MCs levels were dependent on exposure concentration. Clinical studies demonstrated that exposure to MCs exposure can severely impair the nervous system.

2.3 Genotoxicity

Studies on the genotoxicity of MCs have mainly focused on purified MCs and MC extracts. In the Ames assay, purified MC-LR showed negative results. Exposure to MC-LR (0.01 μg/mL) can cause DNA strand breaks in human hepatoma cell line HepG2. Lankoff et al (2006) found that after CHO cells treated with MC-LR (10 μg/ml), the mitosis spindles became abnormal. For MC extracts, Ding et al (1999) showed that MC extracts could cause DNA damage and had extremely high mutagenicity. Generally, the genotoxicity of MC extracts is higher than with purified MCs, which means that other impurities in MC extracts and MCs have synergistic effects on genotoxicity.

2.4 Carcinogenicity

Using a Solt-Farber Model to study the carcinogenicity of MC-LR showed that MC-LR could enhance the expression of positive foci for the placental form of glutathione S-transferase Pi (GSTPi) in rat liver, which was initiated with diethylnitrosamine, and angiomatoid nodules appeared in the liver, indicating MC-LR is a liver tumor promoter. After initiation by smearing dimethylbenzanthracene on the skin, Swiss rats were given drinking water with MC extracts; their skin tumor weight was appreciably higher than in the control group. Other research showed that with injection of azoxymethane, C57Bl/6J rats were given drinking water with MC extracts, and visible angiomatoid nodule appeared in their colons. This evidence demonstrated that MCs have tumor-promoting effects.

2.5 Reproductive and Developmental Toxicity

Recent studies have verified that MCs can accumulate in eggs, and may be transferred to offspring. Chronic exposure to MCs may be toxic to the reproductive system in male rats. This is because that MC-LR may lead to testicular damage through alteration of oxidative stress. Studies showed that MCs can be detected in spermatogenous cells and Sertoli cells, but not detected in Leydig cells, which means spermatogenous cells and Sertoli cells may be the target cells of MCs.

MCs can pass through the placental barrier and cause damage in kidney and liver, increasing incidence of malformed infants. MCs can lead to degeneration, dropsy and interstitium loosening in all placental cells. MCs can directly injure the placental barrier and enter into the embryo, which affects embryonic development and leads to visceral growth damage. MC-LR's toxicity can be passed on to offspring through parental transmission. In adult zebrafish, exposure to MC-LR can cause serious developmental toxicity, including growth inhibition (decrease of body weight and length) and disorder of the F1 immune system.

3. Epidemiological Evidence of the Effects of MCs on Human Health

3.1 Hepatotoxicity

Liu et al (2017) conducted a cross-sectional study in the Three Gorges Reservoir Region, which demonstrated that organ recovery systems (Ors) for abnormal aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in a hepatitis-B virus (HBV) exposed population were not significantly different from those in HBV & aflotoxin B1 (AFB1) or in the HBV&MC-LR exposure population but were significantly higher in the HBV&AFB1&MC-LR exposure population, indicating that MCs may have the potential to increase the risk of liver injury induced by combined exposure to HBV and aflatoxin. In addition, chronic exposure to MCs may be associated with liver damage in children, AST and ALP levels were significantly higher in high-microcystin-exposed children than in low-exposed and unexposed children. Yu et al (2010) found that for residents who lived around Dianshan Lake for over 10 years, the activity of AST, lactate dehydrogenase (LDH) and gamma-glutamy transferse (GGT) in their serum were all higher than in the control group (factory workers whose working and living environments were not affected by Dianshan Lake).

3.2 Carcinogenicity

Epidemiological studies revealed that chronic exposure to MCs through drinking water and liver cancer were positive correlated. But a cohort study conducted in Florida did not confirm this correlation. Existing cohort studies about the carcinogenicity of MCs mainly focus on ecological epidemiology, and they concentrate on China. Epidemiological studies demonstrated that Haimen, Qidong in Jiangsu Province, Tongan in Fujian Province, Fusui, and Suiyuan in Guangxi Province of the Southeastern coastal areas are high incidence areas of liver cancer. Some residents in these cities have been exposed to drinking pond-ditch water contaminated with MCs for a long time, and the incidence of primacy liver cancer is positively correlated with MC levels. Yu et al (2001) found that compared to a population drinking deep well water, a population drinking ditch-pond water is eight times more likely to get liver cancer. Chronic exposure to drinking water with MCs less than 0.3 μg/L will cause liver damage and increase liver enzymes, leading to a high incidence of liver cancer.

Studies conducted in Haimen and Qidong showed that for residents who have been exposed to drinking pond-ditch water with MC contamination, the relative risk of liver cancer was 1.96 and 2.39 times larger than those who drink well water and tap water. Zheng et al. (2017) revealed that there was a positive interaction between MC exposure and HBV infection on liver cancer risk, and the relative excess risk for a population with MC exposure combined with HBV infection exceeded the multiplication of the relative excess risk for MC exposure and HBV infection alone. These results demonstrated that MCs in drinking water is a major risk factor for liver cancer.

Besides liver cancer, MCs and digestive tumors such as gastric cancer and colorectal cancer are positively correlated. Studies showed that MCs in drinking water positively correlated with mortality from male stomach cancer and the standardized mortality of male cancers overall. In Haining, Zhejiang Province, the incidence of colorectal cancer correlated with concentration of MCs in local surface water.

4. Guidelines for drinking-water MCs

The most important toxins that result in a health risk to humans are the neurotoxic alkaloids, microcystins, and cylindrospermopsins. However, the only cyanobacterial toxins evaluated for health risk by the WHO are the microcystins, which are proved to trigger acute liver injury and are tumor promoters. A guideline limit value for microcystin-LR of 1μg/L has been determined by WHO based on health risk assessment. Both developed and developing countries have adopted this limit value as their legislation and national standard for drinking water. However, this toxin comes from specific algal species such as blue cyanobacteria. When the dominant algal species in the water changes, other toxins such as cylindrospermopsin and neurotoxic alkaloids may be important factors inducing health risk. However, there is no limit value for these toxins although their potential adverse effects are high. For instance, cylindrospermopsin is cytotoxic and genetoxic, and is also thought to be a potential carcinogen. Thus monitoring of algal toxins in drinking water and toxicological research based on exposure levels in populations are urgently needed to provide comprehensive data for health risk assessments.

5. Health risks of cyanobacteria toxin contamination in rural areas

We have known that many rivers, lakes, and reservoirs in China are polluted by cyanobacteria due to water body eutrophication, but there is no thorough or comprehensive information about the situation of cyanobacterial pollution and algal toxins. Most data on cyanobacterial and algal toxins contamination in drinking water come from urban water supply systems. Monitoring data about cyanobacterial toxins are not available for rural drinking water. Key reasons that caused this situation are lack of sensitivity in detection instumentation, technical conditions and capabilities. One opinion is that water quality in rural areas is better than that of urban areas, thus the issues of cyanobacterial pollution in rural areas have not attracted much attention for a long time. Accumulating evidence has demonstrated that water pollution in the rural of China may be more serious. Moreover, in rural areas of China, several important factors that cause water body eutrophication are widespread. For instance, lack of infrastructure sanitation that arises from sewage and wastewater discharge without being effectively controlled. Agricultural fertilizer use increases nutrients into water bodies. High population density in rural areas leads to significant increase of domestic sewage discharge. Agricultural run-off and storm water result in nutrients spreading to ponds, lakes, reservoirs, and rivers that promote cyanobacterial proliferation and lead to frequent water blooms. Indeed, two decades ago epidemiological investigations conducted in rural area of China suggest that MCs may be responsible for the high incidence of liver cancer in populations (Ueno et al., 1996). Therefore, the issues of cyanobacterial toxin contamination and its health risk in rural areas have been, and continue to be, ignored.

It is well known that ground water is widely used as a drinking water source in rural China due to surface water pollution and a significant reduction in its water volume. In many rural areas, residents prefer to directly draw ground water as drinking water without taking any treatment because of low cost and easy access; this approach may increase health risk and cause health issues especially in water polluted by different sources. In recent years, severe water pollution-inducing water borne diseases and cancer have been widely reported by both scientific publications and the media. For example, the Huai River Basin is one of the most seriously polluted areas in China, both surface water and ground water are contaminated by industrial and agricultural run-off and activities. Owing to soaring pollution in this area, eutrophication and algal blooms have often occurred in recent years. An epidemiological investigation demonstrated that high cancer mortality in the Huai River Basin of China was associated with serious pollution (Wan, 2009). Eutrophication and algal blooms occurring in surface water subsequently lead to the formation of cyanobacterial toxins, which may influence the quality of ground water. This issue has been of great concern to the academic community for decades. An investigation has demonstrated that microcystins (MCs) can be found in ground water even if no cyanobacterial cells existed, suggesting that MCs can leach from other water bodies through the soil. A key project supported by the Ministry of Science & Technology of the People's Republic of China confirmed that MC contamination in ground water originated from rivers, causing potential health risks on populations who drink ground water directly.

It is worth noting that China has begun to advocate disinfection of drinking water in rural areas. As we know, unintended disinfection by-products (DBPs) are formed during the water treatment processes. DBPs and cyanobacterial toxins inevitably exist together in drinking water, and human populations may be exposed to DBPs and cyanobacterial toxins while drinking. Therefore, their combination is likely to bring a potential health risk for humans. Our previous study provides evidence that combined exposure to MX (a representative of the DBPs) and microsytin-LR exacerbates genotoxicity in CHO cells through oxidative stress, indicating that the issues of drinking water safety in rural areas of China need much more concern.

6. How to control algal pollution hazards

There is no doubt that concentrating on water source conservation is essential for providing healthy drinking water. Effectively controlled water pollution, reduced sewage discharge, and strengthened wastewater treatments are pivotal measures to ensure the safety of drinking water. Generally, local governments and companies should increase investment in environmental pollution control and ecological protection. Improvements of infrastructure sanitation will completely reduce environmental pollution and water quality deterioration caused by pollution. With increased pollution pressure and shortage of surface water, ground water is getting more and more used as a raw water source in the rural areas of China. Importantly, the strategy of using ground water as a drinking water source must be set on the premise that it is not contaminated and does not contain trace elements such as fluoride and arsenic that are harmful to health. In view of the importance of monitoring water quality on management, routinely monitoring and evaluating water quality will serve in a critical manner to provide knowledge on the situation of water pollution. Local government in rural areas is encouraged to provide health education training for their staff involved in health, water supply and environmental management, which raises their awareness about the risks of drinking water containing high concentrations of cyanobacteria. Additionally, capacity building in environmental pollution control, water quality monitoring and management, and health risk assessment need to be gradually improved through training and public health practice. The above mentioned strategies will be vital to ensure public health either in urban or in rural areas of China.

Acknowledgement

This research was supported by grants from National Key R&D Program of China (No.2017YFC1600200), National Natural Science Fund Committee (No.81273035, 81773379 & 81630088), Shanghai Municipal Health Bureau Three Years Action Plan (No. 08GWD14), and Leadership Project (No. 2017) and Natural High-Technology R&D 863 Program (No.2013AA065204).

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